Acta Oceanol. Sin., 2017, Vol. 36, No. 10, P. 31–38
DOI: 10.1007/s13131-017-0976-4
http://www.hyxb.org.cn
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Stocking density effects on growth and stress response of juvenile turbot (*Scophthalmus maximus*) reared in land-based recirculating aquaculture system

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Received 24 May 2016; accepted 2 July 2016

Abstract

Stocking density is widely recognized as a critical factor in aquaculture and a potential source of long-term stress. The influence of stocking density on growth and stress response of juvenile turbot (*Scophthalmus maximus*, ~3–75 g, initial to final weight) was examined in fish held under low (LD, ~0.21–5.31 kg/m^2, initial to final density), medium (MD, ~0.42–10.81 kg/m^2) and high stocking density (HD, ~0.63–14.27 kg/m^2) for 120 days in a recirculating aquaculture system (RAS). In this trial, the growth curve for weight of juvenile turbot in RAS, all fitted by the Schnute model. No significant difference was found in growth performance among the three densities until at the final sampling (Day 120). The final weight and body weight increase (BWI) in the HD group were significantly lower than in other groups (P<0.05, weight: (75.83±2.49) g, (75.39±2.08) g, (65.72±2.86) g and BWI: (2 436.12±28.10)%, (2 421.29±4.64)%, (2 097.88±20.99)% in LD, MD and HD groups, respectively). Similarly, the specific growth rate (SGR), feed conversion ratio (FCR) and coefficient of variation for weight (CV_w) were adversely affected by high stocking density (P<0.05). However, there was no difference in survival and Fulton's condition factor (K) of turbot among the different groups. Physiological analyses demonstrated a clear increase in the plasma cortisol level and an obvious decrease in growth hormone (GH) concentration in the HD group on Day 120 (P<0.05). There was no significant effect of stocking density on plasma glucose, Cl^- and protein levels. All these findings would provide a reference for selecting the optimal stocking density of juvenile turbot in RAS.

Key words: growth performance, recirculating aquaculture system, *Scophthalmus maximus*, stress physiology, stocking density

Citation: Liu Baoliang, Jia Rui, Zhao Kuifeng, Wang Guowen, Lei Jilin, Huang Bin. 2017. Stocking density effects on growth and stress response of juvenile turbot (*Scophthalmus maximus*) reared in land-based recirculating aquaculture system. Acta Oceanologica Sinica, 36(10): 31–38, doi: 10.1007/s13131-017-0976-4

1 Introduction

In the last decade, aquaculture based on industrial rearing systems together with intensive fish farming has become the focus of interest. In intensive aquaculture systems, stocking density is an important factor that governs productivity (Riche et al., 2013) because it is often found to influence growth, survival, water quality, fish welfare and health (Yi and Lin, 2001; Bolasina et al., 2006; Salas-Leiton et al., 2010; Biswas et al., 2013). High stocking density may induce chronic stress associated with deterioration in water quality or adverse social interactions, and this can result in negative biochemical changes (Montero et al., 1999; Bolasina et al., 2006). This can affect the rate and efficiency of feeding and digestion, and subsequently growth (Rowland et al., 2006).

Turbot (*Scophthalmus maximus*) is farmed in Europe and China, and in commercial operations, 100 g turbot can be reared at a density of 25 kg/m^2, whereas stocking densities generally range from 30 kg/m^2 to 40 kg/m^2 for larger fish (Aksungur et al., 2007; Baer et al., 2011). However, this management strategy ignores the possible negative impact of high densities on fish growth.

Recirculating aquaculture system (RAS) is an important model in global aquaculture industry, given its cost-effective, environment-friendly and product safety features as well as easy regulation of water quality (d'Orbcastel et al., 2009). Land-based RAS farming has been developed on an industrial scale by overcoming the technical challenges. It is used in fish farming, including sea bass (*Dicentrarchus labrax*) (Deviller et al., 2005), cobia (*Rachycentron canadum*) (Resley et al., 2006), rainbow trout (*Oncorhynchus mykiss*) (Mansfield et al., 2010) and Atlantic salmon (*Salmo salar*) (Burr et al., 2012). The relationships between stocking density and growth performance, metabolism, immunity and

Foundation item: The National Natural Science Foundation of China under contract Nos 31402315 and 31240012; the Modern Agriculture Industry System Construction of Special Funds under contract No. CARS-50-G10; the Key R & D Program of Jiangsu Province under contract No. BE2015328; a foundation from the Key Laboratory of Mariculture & Stock Enhancement in North China's Sea, Ministry of Agriculture, China.

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well-being of these fish were reported in RAS (Heinen et al., 1996; Sammouth et al., 2009; Liu et al., 2014). However, in turbot, few studies have focused on the effects caused by stocking density in a common commercial model of RAS. Lab-scale studies evaluating the impact of stocking density on growth performance might not easily scale up to the larger commercial environment. Therefore, in this study, we evaluated the effects of stocking density on the growth and stress physiology of juvenile turbot under production conditions using the common commercial model of RAS. These results enhance the management of RAS-cultured turbot in determining the optimal stocking density.

2 Materials and methods

2.1 *Experimental facilities*

The study was conducted in a commercial land-based RAS at Shandong Oriental Ocean Sci-Tech Co., Ltd (Shandong, China). The RAS consisted of ten octagonal tanks, ten whirl-separators, a filter screen, a foam-separation unit, a bio-filter section consisting of four separate bio-filters in parallel (each 35 m^3), a UV sterilizer, and a DO regulating tank (Fig. 1). Each rearing tank (30 m^2 and 1.1 m in depth) was equipped with a mixture of water supersaturated with oxygen from an oxygen cone. The oxygen content and water level (0.5–0.55 m in depth) in the fish tanks were monitored via the RAS computer. The volume of the whirl-separator was approximately 300 L, which was adequate to collect most of the feces. Water (16–18°C) was pumped from a depth of 20 m in the Laizhou Bay of China, mechanically filtered by two sand filters (5 μm filtration) and UV-sterilized before entering the RAS units. Water flows through standpipes covered with 2.0 cm screen to rearing tank were about 16 m^3/h, and no more than 10% volume of water in the system was displaced with fresh seawater every day during culture. The temperature in each tank varied slightly throughout the day, but in all cases was maintained at (18±1)°C throughout the trial. The photoperiod was maintained at 12 h light/12 h dark using fluorescent light banks. Further, the nitrification function in bio-filters was already established in the RAS prior to trial.

2.2 *Experimental design and management*

Turbot were obtained from this facility and reared in the RAS for 15 d to acclimatize to the experimental conditions. The fish (average individual weight (2.99±0.21) g) were reared under three stocking densities: low, 2 200 fish per tank ((0.21±0.01) kg/m^2); medium, 4 400 fish per tank ((0.42±0.02) kg/m^2); and high, 6 600 fish per tank ((0.63±0.05) kg/m^2), and tested in triplicate. In total, 39 600 fish were investigated in nine rearing tanks of a commercial land-based RAS (the tenth tank is empty) and cultured under experimental conditions for 120 days. No differences in mean initial weight and coefficient of variation (CV) in initial weight were found among the three densities. The turbot were fed a commercial-pellet diet (Ningbo Tech-Bank Co., Ltd, Zhejiang China), which contained 52% crude protein, 12% crude lipids, 16.0% crude ash, 3.0% crude fiber, 12% water, 5% Ca, 0.5% P, ⩾ 2.3% lysine, and ⩽3.8% sodium chloride. The fish were fed at ration of approximately 2.5% of the tank biomass which divided into four meals daily by hand (06:30, 11:30, 16:30 and 21:30). The feed rations were adjusted based on feeding behavior, weight as well as the practical production experience. The ration of 2.5% was chosen because it was close to satiation, allowed an optimum growth and no excess feed appeared on the bottom of the tanks. Fish were not fed on the sampling day to minimize handling stress.

2.3 *Water quality monitoring*

Temperature, dissolved oxygen (DO), pH, and salinity were measured daily using a YSI-556 (YSI Incorporated, Yellow Springs, Ohio, USA). Orthophosphates (PO_4-P), total ammonium nitrogen (TAN), nitrite-nitrogen (NO_2-N), and chemical oxygen demand (COD) were analyzed using a standard method (Chen, 2006) every ten days.

2.4 *Growth parameters and survival*

Dead fish in all tanks were recorded daily to evaluate the survival rate over the entire study period. Fish growth was evaluated biometrically every 9 d, by randomly measuring the individual weight and standard length of 200–400 fish in each tank (Garcia et al., 2013), to calculate stocking density, specific growth rate (SGR), feed conversion ratio (FCR), Fulton's condition factor (K), coefficient of variation for weight (CV_w), and percentage of covered area (PCA). At the end of this trial, the final body weight increase (BWI) were also calculated. These growth parameters were calculated as follows:

Survival=100×final number of fish/initial number of fish;

BWI=100×(final weight–initial weight)/initial weight;

SGR=100×(ln(final weight)–ln(initial weight))/number of days;

FCR=food consumed/biomass increment;

K=100×weight/length^3;

CV_w=100×weight standard deviation/mean weight;

Stocking density=(N×W_t)/A;

PCA=N×(A_f/A)×100;

A_f (m^2)=(102.5×weight+3 595.0)×10^{-6}, when weight is Less than

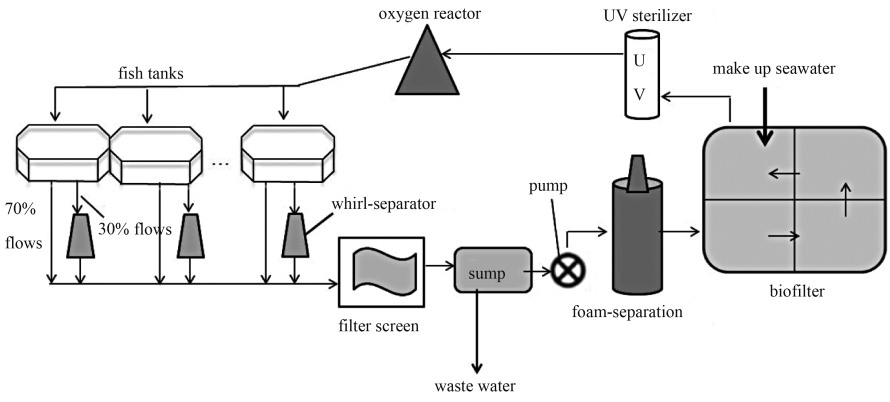

Fig. 1. Schematic diagram of experimental land-based RAS. Water flows from rearing tanks-whirl-separator (30% flows)-filter screen-sump-pump-foam-separation-biofilter-UV sterilizer-oxygen reactor-rearing tanks.

100 g (Irwin et al., 1999);
where N is number of individuals, W_t is average weight (g) at sampling day, A is the bottom area of the fish tank, and A_f is the area of the fish body.

2.5 *Fish sampling and biochemical assays*

The fish were starved for 24 h before sampling for biochemical parameters on Days 30, 60, 90 and 120. Twenty fish were randomly netted from each tank, immediately anesthetized in 0.05% tricaine methane sulfonate (MS-222, Sigma Diagnostics INS, St. Louis, MO, USA). Blood samples were obtained from the caudal vein using heparinized syringes. The plasma was separated by centrifugation (5 000 r/min, 4°C, 15 min) and stored at –80°C for analysis of cortisol, growth hormone (GH), glucose, chloride ion (Cl^-) and protein. Plasma cortisol was determined using radioimmunoassay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) available on market as previously described methods (Liu et al., 2015). Plasma GH (μg/L) were measured using a commercially available ELISA kit (Mlbio, Shanghai, China) as previously described by Drennon et al. (2003). Plasma glucose and chloride (mmol/L) were analyzed using an i-STAT Portable Clinical Analyzer (Abbott Inc, Illinois, USA) with EC8+ disposable cartridges (Abbott Laboratories, Illinois, USA). Protein concentrations in plasma was determined by the Bradford method (Bradford, 1976), using bovine serum albumin as a standard; final number of fish=initial number of fish–dead fish (not include the loss in sampling).

2.6 *Statistical analysis*

The differences among the different groups were analyzed using one-way analysis of variance (ANOVA) and $P<0.05$ was taken as statistically significant. All experimental treatments of growth performance, and plasma parameters were performed in triplicate. Data were expressed as mean±SD. Linear regression was used to analyze the possible effects of stocking density on SGR. A third degree polynomial was used to compare the density and FCR (Björnsson et al., 2012). All statistical analyses were carried out using SPSS Version 18.0 software (SPSS Inc, Chicago, IL, USA).

3 Results

3.1 *Water quality*

During the course of experiment, the water temperature, DO, pH and salinity were maintained at (18±1)°C, (8±1) mg/L, 7.9±0.3, and 27.3±1, respectively, in all tanks. There were no significant differences among the three stocking densities in NO_2-N and PO_4-P levels (Fig. 2). Between Days 0 and 120, the TAN concentration showed an increasing trend, with no differences among the three stocking densities before Day 100, while it was significantly higher in the HD group than in the LD group at and after Day 100 ($P<0.05$, Fig. 2). For the period including Days 0 to 110, the variation in COD concentration was similar in all tanks, however, a significant increase occurred in HD group on Day 120 compared with the LD group ($P<0.05$, Fig. 2).

3.2 *Growth performance*

During the experiment, no disease outbreak or other signs of disease were observed. Survival was extremely high (>99.5%) in all treatments with no significant differences (Tables 1 and 2). The stocking density and PCA increased gradually with culture time (Figs 3a and b). The final densities (Day 120) were, respectively, (5.31±0.48), (10.81±1.04) and (14.27±1.21) kg/m^2 for LD, MD and HD. The final proportion of the tank bottom covered by turbot (PCA) in the LD, MD and HD were (82.81±0.65)%, (165.49±0.12)% and (226.18±1.71)%, respectively. There were no

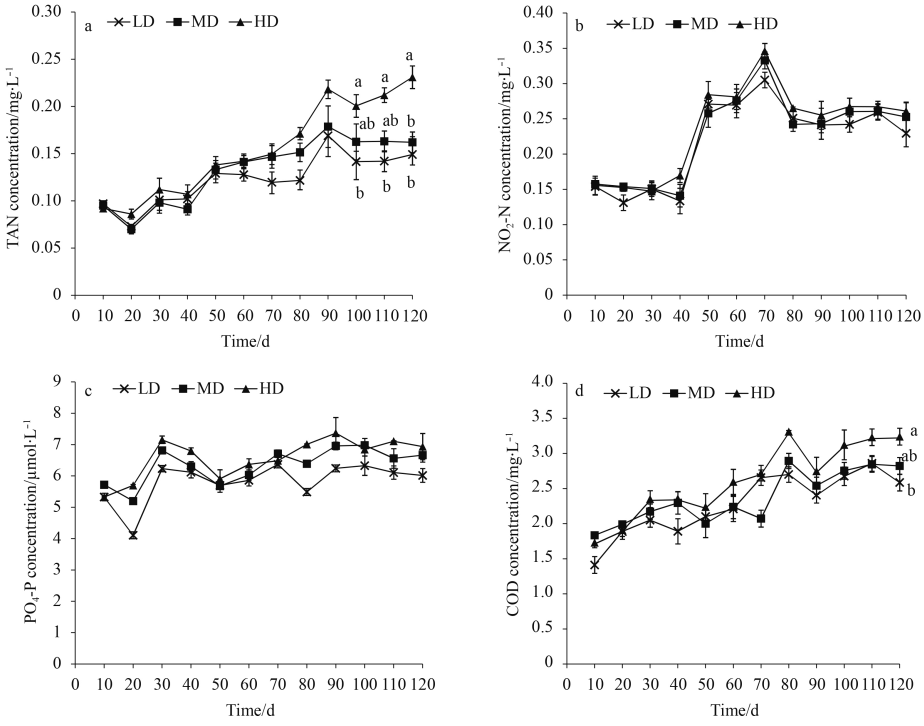

Fig. 2. Key water quality parameters in RAS. Data are presented as mean±SD (n=3 duplication of each density group). A different letter at the same sampling time indicates significant differences among the three stocking densities (LD represents low density, MD medium density and HD high density; $P<0.05$).

Table 1. Initial weight, final weight, initial density and final density, initial percentage of covered area (PCA), final body weight increase (BWI) and survival of turbot reared in different stocking densities

Variable	Density treatment		
	2 200 fish per tank (LD)	4 400 fish per tank (MD)	6 600 fish per tank (HD)
Initial weight/g	2.99±0.16	2.99±0.16	2.99±0.26
Final weight/g	75.83±2.49[a]	75.39±2.08[a]	65.72±2.86[b]
Initial density/kg·m^{-2}	0.21±0.01	0.42±0.02	0.63±0.05
Final density/kg·m^{-2}	5.31±0.48	10.81±1.04	14.27±1.21
Initial PCA/%	28.61±1.42	57.22±1.43	85.83±2.44
Final PCA/%	82.81±0.65	165.49±0.12	226.18±1.71
Final BWI/%	2 436.12±28.10[a]	2 421.29±4.64[a]	2 097.88±20.99[b]
Final survival/%	99.33±0.03	99.66±0.08	99.52±0.13

Note: Values are expressed as mean±SD in triplicate (n=3 duplication of each density group). For each row, data with different letters as superscripts are significantly different (P<0.05).

Table 2. Survival, Fulton condition factor (K), special growth rate (SGR) and feed conversion ratio (FCR) and of juvenile turbot in 120-d old cultures at different stocking densities in RAS

Time/d		LD	MD	HD
Survival/%	30	99.8±1.79	99.9±1.69	99.8±1.02
	60	99.7±1.08	99.8±1.03	99.8±1.08
	90	99.6±1.16	99.7±1.86	99.6±2.13
	120	99.5±2.19	99.6±2.98	99.6±3.76
K	0	3.03±0.11	3.02±0.12	3.04±0.15
	30	3.30±0.10	3.39±0.14	3.43±0.18
	60	3.76±0.21	3.72±0.11	3.81±0.12
	90	3.39±0.10	3.28±0.12	3.27±0.11
	120	3.63±0.08	3.58±0.07	3.52±0.10
SGR in each growth period/%·d^{-1}	Period 1/Days 0–30	3.23±0.07	3.13±0.07	3.10±0.02
	Period 2/Days 31–60	3.20±0.06	3.19±0.05	3.15±0.02
	Period 3/Days 61–90	2.01±0.08	2.08±0.03	1.95±0.13
	Period 4/Days 91–120	2.35±0.11[a]	2.35±0.07[a]	2.09±0.04[b]
FCR in each growth period	Period 1/Days 0–30	0. 64±0.024	0.62±0.039	0.64±0.012
	Period 2/Days 31–60	0.70±0.019	0.73±0.005	0.76±0.033
	Period 3/Days 61–90	0.76±0.025	0.75±0.070	0.75±0.024
	Period 4/Days 91–120	0.82±0.008[a]	0.81±0.011[a]	1.06±0.065[b]

Note: Values are expressed as mean±SD in triplicate (n=3 duplication of each density group). For each row, means on the same day denoted with different letters as superscripts are significantly different (P<0.05).

significant differences in individual weight and standard length associated with treatments from Day 0 to Day 110, while both the parameters in LD and MD were obviously higher than in HD on Day 120 (P<0.05, Figs 3c and d). At the end of the trial, significantly lower SGR and significantly higher FCR were observed in HD compared with LD and MD (P<0.05, Table 2). In the study, the Fulton's condition factor (K) did not differ.

The CV_w was not significantly different among the three density groups until the final Day 120 when the CV_w of the HD group was clearly larger than in the MD and LD groups (P<0.05, Fig. 4).

In the four growth periods, a significant negative correlation between stocking density and SGR was observed (Fig. 5a), although only 69.50% of the variation was explained by the correlation (R^2=0.695, N=36, P<0.01). There was only a minor increase in FCR with density increasing from 0.5 to 11 kg/m^2 but a growing increase from 10 to 14 kg/m^2 (Fig. 5b). In addition, we also found the growth curve for weight-for-age data for juvenile turbot reared at three different densities in RAS, fitted by the Schnute model (Fig. 6).

3.3 *Physiological parameters*

The physiological parameters between the treatment densities at different time points in the experiment are listed in Table 3. Post-hoc analysis demonstrated a higher level of plasma cortisol under HD treatment compared with LD treatment on Day 120. Conversely, the plasma GH concentration in HD and MD treatment was evidently lower than in LD treatment. In addition, the plasma glucose, Cl^- and protein levels did not vary between groups during the rearing experiment.

4 Discussion

In the RAS, water quality was maintained at safe levels recommended for turbot, with the outlet O_2 concentration always above 6 mg/L, TAN concentration lower than 0.25 mg/L, and NO_2^--N concentration under 0.4 mg/L (Poxton and Allouse, 1987; Skøtt Rasmussen and Korsgaard, 1996; Person-Le Ruyet et al., 1997; Aubin et al., 2006). Although the values of TAN and COD were significantly higher in HD than in LD between Days 100 and 120 of the trial, the variations did not affect turbot negatively (Aubin et al., 2006; Song et al., 2012).

In the present study, the survival was excellent and was not significantly affected by stocking density. Although many studies reported an inverse correlation between survival and density (Yi and Lin, 2001; Hitzfelder et al., 2006; Rowland et al., 2006), a few studies fail to report any significant effect of density on survival in teleosts (Webb et al., 2007; Laiz-Carrión et al., 2012; Riche et al.,

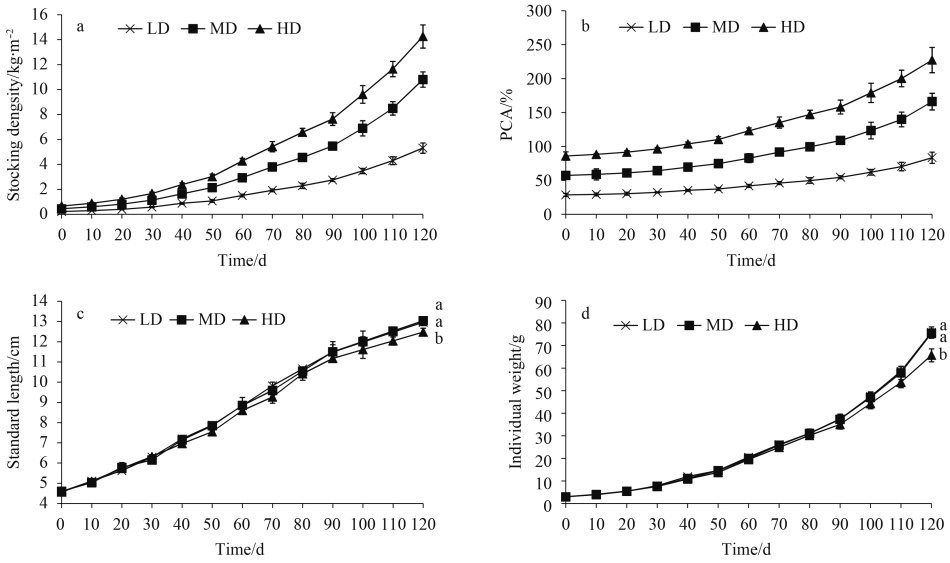

Fig. 3. Changes in stocking density (a) percentage of covered area (PCA, b), standard length (c) and individual weight (d) of RAS-cultured juvenile turbot with different experimental treatments. Data are present as mean±SD. A different letter on the same day indicates significant differences in the three stocking densities (P<0.05).

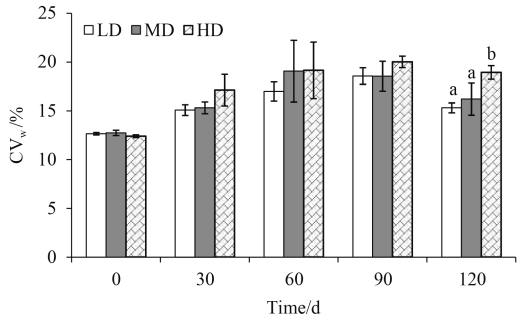

Fig. 4. The coefficient of variation for weight (CV_w) on Days 0, 30, 60, 90 and 120 for each stocking density of RAS-cultured juvenile turbot. Data are presented as mean±SD (n=3 duplication of each density group). A different letter on the same day indicates significant differences in the three stocking densities (LD represents low density, MD medium density and HD high density; P<0.05).

2013). Stocking density affected the growth in fish, but the correlation between the two parameters may not be uniformly linear positively or negatively in a given species (Irwin et al., 1999). Studies with cobia (*Rachycentron canadum*) demonstrated a direct inverse relationship between growth and density (Webb et al., 2007). However, conflicting results showed an increased growth rate under high density in juvenile silver perch (*Bidyanus bidyanus*) (Rowland et al., 2006). In the current study, stocking density affected the values of individual weight and standard length, SGR and BWI at the end of the trial in RAS, suggesting that when stocking densities increased to a certain level (e.g., exceed to 14.27 kg/m^2) could negatively affected the growth performance of flatfish populations (Irwin et al., 1999; Sánchez et al., 2013). The FCR was remarkably stable and low during the first three months at all three densities, but it increased rapidly in the high-density group during the final growth period. Similar findings were reported in juvenile cod and rockfish (*Sebastes schlegeli*) (Björnsson et al., 2012; Hwang et al., 2014). In addition, the growth curves of juvenile turbot reared at three densities in RAS fitted by the Schnute model, were consistent with the growth model in turbot reported by Baer et al. (2011).

Notably, weight heterogeneity (often expressed as the CV_w) has been suggested as an indicator of the social environment within fish populations, where a higher CV_w may be attributed to increased stocking density and indicate inter-individual competition within the fish group (Irwin et al. 1999; North et al. 2006; Rowland et al. 2006). A few species such as piabanha (*Brycon insignis*) (Tolussi et al., 2010), pearl spot (*Etroplus suratensis*) (Biswas et al., 2013) and European sea bass (*Dicentrarchus labrax*) (Di Marco et al., 2008), showed homogeneous CV_w in different stocking densities. In this study, the results indicated a high-

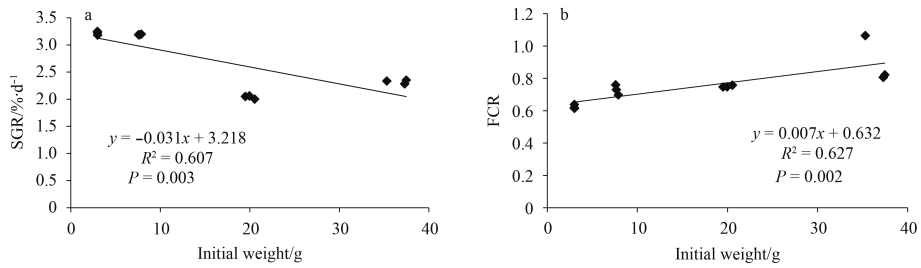

Fig. 5. Relationships between relative growth rate and stocking density (a) and relationships between the feed conversion ratio (FCR) and stocking density (n=36) (b).

Table 3. Physiological parameters of juvenile turbot in 120-d old cultures at different stocking densities in RAS

Parameter	Density	Time/d			
		30	60	90	120
Plasma cortisol/μg·L^{-1}	LD	1.29±0.16	1.26±0.16	1.28±0.08	1.16±0.04^{a}
	MD	1.18±0.05	1.20±0.10	1.35±0.14	1.36±0.10^{a}
	HD	1.34±0.23	1.37±0.06	1.37±0.17	1.72±0.14^{b}
Plasma GH/μg·L^{-1}	LD	21.6±1.68	18.5±1.33	18.5±1.68	18.3±0.31^{a}
	MD	20.2±2.44	17.5±3.05	18.9±2.04	14.9±1.72^{b}
	HD	19.9±0.47	16.1±1.55	17.9±2.1	14.4±0.68^{b}
Plasma glucose/mmol·L^{-1}	LD	2.19±0.38	1.20±0.16	2.07±0.11	1.43±0.29
	MD	1.99±0.44	1.27±0.16	2.25±0.28	1.40±0.13
	HD	2.06±0.17	1.23±0.35	2.0±0.06	1.41±0.11
Plasma Cl^{-}/mmol·L^{-1}	LD	127.8±9.81	120.9±2.89	120.1±3.35	116.5±2.72
	MD	120.1±4.21	121.8±4.57	124.8±3.69	115.5±2.58
	HD	130.5±2.87	122.8±3.76	116.7±1.35	124.0±5.73
Plasma protein/g·L^{-1}	LD	21.5±1.86	21.5±1.4	23.5±1.52	26.1±3.58
	MD	23.5±2.29	22.9±2.07	26.1±0.94	22.4±0.87
	HD	23.4±2.99	20.4±0.33	23.1±0.94	22.9±1.17

Note: Data are expressed as mean±SD (n=3 duplication of each density group). For each column, means on the same day with different letters as superscripts are significantly different (P<0.05).

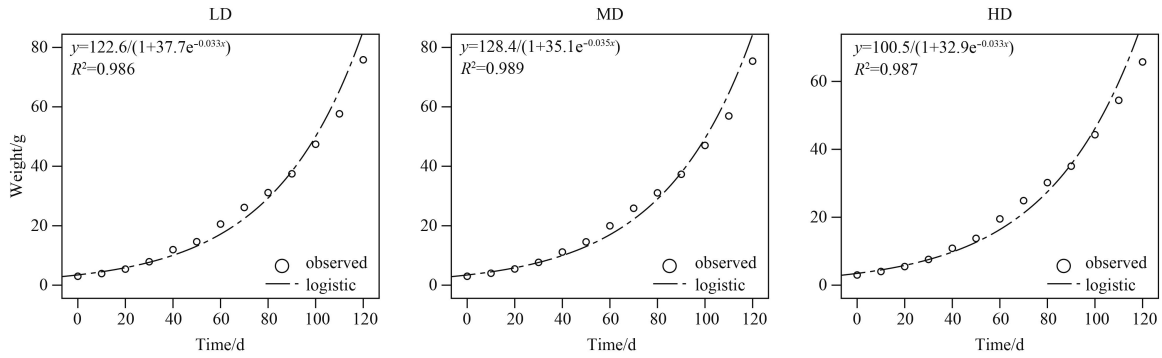

Fig. 6. Growth curve representing weight-for-age data in juvenile turbot reared at three different densities (LD represents low density, MD medium density and HD high density), fitted by the Schnute model (n=240). A sub-sample of 80 randomly selected fish from each replicate at the three treatment densities.

er CV_w in HD treatment at the conclusion of the experiment suggesting that the fish weight in HD treatment varied drastically. Similar data were also reported in turbot cultured in small experimental RAS (Irwin et al., 1999).

Plasma cortisol levels may be an effective indicator of primary stress response in fish (Ellis et al., 2012). High stocking density has been considered as a chronic stressor producing a chronic elevation of plasma cortisol (Ellis et al., 2002; Bolasina et al., 2006). The fish reared under high density presented significantly higher cortisol levels on Day 120, implying that high density produced a stress response. This effect has been described in different species, including Japanese flounder and Senegalese sole (Bolasina et al., 2006; Salas-Leiton et al., 2010; Costas et al., 2013). Further, glucose is an important stress factor, which might be up-regulated with the increase in the cortisol level (van Raaij et al., 1996). However, the current study showed that glucose level was not affected by stocking density in turbot, which was consistent with reports in sea bass (*Dicentrarchus labrax*) and juvenile cod (*Gadus morhua*) (Foss et al., 2006; Di Marco et al., 2008). GH has also been associated with stress (Rodríguez et al., 2000; McCormick, 2001). Menezes et al. (2015) observed lower values of GH expression in silver catfish (*Rhamdia quelen*) reared at high density compared with low density. Similar results were displayed in our study with lower GH levels in MD and HD groups at the end of the trial.

5 Conclusions

This study showed that juvenile turbot was efficiently cultured on a commercial scale in RAS. Our findings suggested that a stocking density up to 11.7 kg/m^2 (corresponding to Day 110 in HD group) was not associated with any negative impact on the growth of juvenile turbot. However, the growth performance and the physiological response were adversely affected at a stocking density of 14.27 kg/m^2 (Day 120). Our results provide a reference standard for the selection of stocking densities of turbot in commercial land-based RAS.

References

Aksungur N, Aksungur M, Akbulut B, et al. 2007. Effects of stocking density on growth performance, survival and food conversion ratio of turbot (*Psetta maxima*) in the net cages on the south-eastern coast of the Black Sea. Turkish J Fish Aquat Sci, 7(2): 147–152

Aubin J, Papatryphon E, Van der Werf H M G, et al. 2006. Characterisation of the environmental impact of a turbot (*Scophthalmus maximus*) re-circulating production system using Life Cycle Assessment. Aquaculture, 261(4): 1259–1268

Baer A, Schulz C, Traulsen I, et al. 2011. Analysing the growth of turbot (*Psetta maxima*) in a commercial recirculation system with

the use of three different growth models. Aquacult Int, 19(3): 497–511

Biswas G, Ghoshal T K, Natarajan M, et al. 2013. Effects of stocking density and presence or absence of soil base on growth, weight variation, survival and body composition of pearlspot, *Etroplus suratensis* (Bloch) fingerlings. Aquacult Res, 44(8): 1266–1276

Björnsson B, Steinarsson A, Oddgeirsson M, et al. 2012. Optimal stocking density of juvenile Atlantic cod (*Gadus morhua* L.) reared in a land-based farm. Aquaculture, 356–357: 342–350

Bolasina S, Tagawa M, Yamashita Y, et al. 2006. Effect of stocking density on growth, digestive enzyme activity and cortisol level in larvae and juveniles of Japanese flounder, *Paralichthys olivaceus*. Aquaculture, 259(1–4): 432–443

Bradford M M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72(1–2): 248–254

Burr G S, Wolters W R, Schrader K K, et al. 2012. Impact of depuration of earthy-musty off-flavors on fillet quality of Atlantic salmon, *Salmo salar*, cultured in a recirculating aquaculture system. Aquacult Eng, 50: 28–36

Costas B, Aragão C, Dias J, et al. 2013. Interactive effects of a high-quality protein diet and high stocking density on the stress response and some innate immune parameters of Senegalese sole *Solea senegalensis*. Fish Physiol Biochem, 39(5): 1141–1151

d'Orbcastel E R, Person-Le Ruyet J, Le Bayon N, et al. 2009. Comparative growth and welfare in rainbow trout reared in recirculating and flow through rearing systems. Aquacult Eng, 40(2): 79–86

Deviller G, Palluel O, Aliaume C, et al. 2005. Impact assessment of various rearing systems on fish health using multibiomarker response and metal accumulation. Ecotoxicol Environ Saf, 61(1): 89–97

Di Marco P, Priori A, Finoia M G, et al. 2008. Physiological responses of European sea bass *Dicentrarchus labrax* to different stocking densities and acute stress challenge. Aquaculture, 275(1–4): 319–328

Drennon K, Moriyama S, Kawauchi H, et al. 2003. Development of an enzyme-linked immunosorbent assay for the measurement of plasma growth hormone (GH) levels in channel catfish (*Ictalurus punctatus*): assessment of environmental salinity and GH secretogogues on plasma GH levels. Gen Comp Endocrinol, 133(3): 314–322

Ellis T, North B, Scott A P, et al. 2002. The relationships between stocking density and welfare in farmed rainbow trout. J Fish Biol, 61(3): 493–531

Ellis T, Yildiz H Y, López-Olmeda J, et al. 2012. Cortisol and finfish welfare. Fish Physiol Biochem, 38(1): 163–188

Foss A, Kristensen T, Åtland Å, et al. 2006. Effects of water reuse and stocking density on water quality, blood physiology and growth rate of juvenile cod (*Gadus morhua*). Aquaculture, 256(1–4): 255–263

Garcia F, Romera D M, Gozi K S, et al. 2013. Stocking density of *Nile tilapia* in cages placed in a hydroelectric reservoir. Aquaculture, 410–411: 51–56

Heinen J M, Hankins J A, Weber A L, et al. 1996. A semiclosed recirculating-water system for high-density culture of rainbow trout. The Progressive Fish-Culturist, 58(1): 11–22

Hitzfelder G M, Holt G J, Fox J M, et al. 2006. The effect of rearing density on growth and survival of cobia, *Rachycentron canadum*, larvae in a closed recirculating aquaculture system. J World Aquacult Soc, 37(2): 204–209

Hwang H K, Son M H, Myeong J I, et al. 2014. Effects of stocking density on the cage culture of Korean rockfish (*Sebastes schlegeli*). Aquaculture, 434: 303–306

Irwin S, O'halloran J, FitzGerald R D. 1999. Stocking density, growth and growth variation in juvenile turbot, Scophthalmus maximus (*Rafinesque*). Aquaculture, 178(1–2): 77–88

Laiz-Carrión R, Viana I R, Cejas J R, et al. 2012. Influence of food deprivation and high stocking density on energetic metabolism and stress response in red porgy, *Pagrus pagrus* L. Aquacult Int, 20(3): 585–599

Liu Baoliang, Liu Ying, Liu Ziyi, et al. 2014. Influence of stocking density on growth, body composition and energy budget of Atlantic salmon *Salmo salar* L. in recirculating aquaculture systems. Chin J Oceanol Limnol, 32(5): 982–990

Liu Baoliang, Liu Ying, Wang Xianping. 2015. The effect of stocking density on growth and seven physiological parameters with assessment of their potential as stress response indicators for the Atlantic salmon (*Salmo salar*). Marine and Freshwater Behaviour and Physiology, 48(3): 177–192

Mansfield G S, Desai A R, Nilson S A, et al. 2010. Characterization of rainbow trout (*Oncorhynchus mykiss*) intestinal microbiota and inflammatory marker gene expression in a recirculating aquaculture system. Aquaculture, 307(1–2): 95–104

McCormick S D. 2001. Endocrine control of osmoregulation in teleost fish. Am Zool, 41(4): 781–794

Menezes C, Ruiz-Jarabo I, Martos-Sitcha J A, et al. 2015. The influence of stocking density and food deprivation in silver catfish (*Rhamdia quelen*): a metabolic and endocrine approach. Aquaculture, 435: 257–264

Montero D, Izquierdo M S, Tort L, et al. 1999. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. Fish Physiol Biochem, 20(1): 53–60

North B P, Turnbull J F, Ellis T, et al. 2006. The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 255(1–4): 466–479

Person-Le Ruyet J, Galland R, Le Roux A, et al. 1997. Chronic ammonia toxicity in juvenile turbot (*Scophthalmus maximus*). Aquaculture, 154(2): 155–171

Poxton M G, Allouse S B. 1987. Cyclical fluctuations in ammonia and nitrite-nitrogen resulting from the feeding of turbot, *Scophthalmus maximus* (L.), in recirculating systems. Aquacult Eng, 6(4): 301–322

Resley M J, Webb Jr K A, Holt G J. 2006. Growth and survival of juvenile cobia, *Rachycentron canadum*, at different salinities in a recirculating aquaculture system. Aquaculture, 253(1–4): 398–407

Riche M A, Weirich C R, Wills P S, et al. 2013. Stocking density effects on production characteristics and body composition of market size cobia, *Rachycentron canadum*, reared in recirculating aquaculture systems. J World Aquacult Soc, 44(2): 259–266

Rodríguez L, Begtashi I, Zanuy S, et al. 2000. Development and validation of an enzyme immunoassay for testosterone: effects of photoperiod on plasma testosterone levels and gonadal development in male sea bass (*Dicentrarchus labrax*, L.) at puberty. Fish Physiol Biochem, 23(2): 141–150

Rowland S J, Mifsud C, Nixon M, et al. 2006. Effects of stocking density on the performance of the Australian freshwater silver perch (*Bidyanus bidyanus*) in cages. Aquaculture, 253(1–4): 301–308

Sánchez P, Ambrosio P P, Flos R. 2013. Stocking density affects Senegalese sole (*Solea senegalensis*, Kaup) growth independently of size dispersion, evaluated using an individual photo-identification technique. Aquacult Res, 44(2): 231–241

Salas-Leiton E, Anguis V, Martín-Antonio B, et al. 2010. Effects of stocking density and feed ration on growth and gene expression in the Senegalese sole (*Solea senegalensis*): potential effects on the immune response. Fish Shellfish Immunol, 28(2): 296–302

Sammouth S, d'Orbcastel E R, Gasset E, et al. 2009. The effect of density on sea bass (*Dicentrarchus labrax*) performance in a tank-based recirculating system. Aquacult Eng, 40(2): 72–78

Skøtt Rasmussen R, Korsgaard B. 1996. The effect of external ammonia on growth and food utilization of juvenile turbot (*Scophthalmus maximus* L.). J Exp Mar Biol Ecol, 205(1–2): 35–48

Song Xiefa, Chen Yiming, Peng Lei, et al. 2012. Effects of DO, ammonia and nitrite on growth and metabolism of juvenile turbot. Fishery Modernization (in Chinese), 39(6): 33–39

Tolussi C E, Hilsdorf A W S, Caneppele D, et al. 2010. The effects of stocking density in physiological parameters and growth of the endangered teleost species piabanha, Brycon insignis (*Steindachner*, 1877). Aquaculture, 310(1–2): 221–228

van Raaij M T M, Pit D S S, Balm P H M, et al. 1996. Behavioral

strategy and the physiological stress response in rainbow trout exposed to severe hypoxia. Horm Behav, 30(1): 85–92

Webb Jr K A, Hitzfelder G M, Faulk C K, et al. 2007. Growth of juvenile cobia, *Rachycentron canadum*, at three different densities in a recirculating aquaculture system. Aquaculture, 264(1–4): 223–227

Yi Yang, Lin C K. 2001. Effects of biomass of caged Nile tilapia (*Oreochromis niloticus*) and aeration on the growth and yields in an integrated cage-cum-pond system. Aquaculture, 195(3–4): 253–267

Zhu Chenjian. 2006. Experiment of Seawater Analytical Chemistry (in Chinese). Qingdao: Ocean University of China Press, 40–63